Necrosis Avid Tracer Agent

FIELD OF THE INVENTION

The present invention concerns to the use of phenanthro[1,10,9,8-opqra]perylene-7,14-dione derivatives, and more specifically hypericin or its derivatives, as necrosis or infarct
5   specific agents. The phenanthro[1,10,9,8-opqra]perylene-7,14-dione derivatives can be labeled with a radionuclide, a radiopaque material or a material enhancing the effects magnetic resonance imaging.

BACKGROUND OF THE INVENTION

Non-invasive "hot spot imaging" and localization of necrotic tissue may be helpful for the
10   diagnosis of different disorders. This is especially true in the case of ischemic myocardial injury, where imaging using necrosis avid agents could help in measuring infarct size. This could allow the rapid use of interventions for myocardial salvage through early identification of the acute event, in this way improving the clinical outcome. Also other cardiovascular disorders associated with cardiac cell death could be visualized and diagnosed, such as
15   cardiomyopathies, myocarditis and heart transplant rejection (1).
In Nuclear Medicine, only a limited number of "hot spot imaging" agents have been used or proposed. ^{99m}Tc-pyrophosphate was the first of them and is supposed to bind to necrotic myocardial tissue by targeting calcium phosphate deposited in the mitochondria of infarcted or severely injured myocardial tissue (2). However, ^{99m}Tc-pyrophosphate scintigraphy has
20   never gained widespread use because of its limited diagnostic accuracy and relatively poor stability that may lead to the presence of pertechnetate and so result in imaging of the cardiac chambers. More recently, ^{111}In-labeled murine monoclonal antimyosin Fab antibody fragments were introduced for infarct-avid scintigraphy (3,4). This so-called ^{111}In-antimyosin has a selective affinity for the intracellular heavy chain of cardiac myosin, which is exposed
25   when the integrity of the sarcolemma is lost as a result of cell damage (5,6). It is incorporated in the necrotic myocardium in an inverse relationship to regional flow, with maximum uptake in areas with severe flow impairment, although its uptake is more intense in myocardial infarcts with reperfusion than in those with persistent coronary occlusion (7). It was also found useful for detection of acute or chronic diffuse myocardial damage in allograft
30   rejection in cardiac transplantation (8,9), doxorubicin-induced cardiotoxicity (10), acute myocarditis (11,12) and various cardiomyopathies (13,14). Although ^{111}In-antimyosin was recently approved by the FDA for clinical use, the limitation of approved indications to ischemic heart disease has resulted in cessation of commercial production.

A few years ago, it was observed that the complex of ^{99m}Tc with glucaric acid, a simple dicarboxylic acid sugar, localized in canine reperfused myocardial infarction soon after injection (*15,16*). The tracer agent is supposed to bind to positively charged histones within disintegrated nuclei and reduced subcellular organelle proteins in necrotic myocytes (*17*). It

5   has the advantage of a rapid blood pool clearance, a good target to background ratio, lack of toxicity and antigenicity and is claimed to have a good sensitivity and specificity for detection of early irreversible myocyte injury. On the other hand, its uptake is limited to the first 9 hours after the onset of acute myocardial infarction because of the relatively rapid disintegration of the positively charged histones. Further clinical trials have to reveal its real clinical

10   usefulness.

In the field of Magnetic Resonance Imaging (MRI), it has recently been reported that some porphyrin derivatives that were originally developed as tumor-seeking contrast agents (*18*) have avidity only for nonviable tissues (typically necrosis) instead of viable tumoral cells and could therefore be re-categorized as necrosis-avid contrast agents (NACAs) (*19-21*). The

15   targetability of these agents for necrosis has elicited novel applications for MRI visualization of acute myocardial infarction (*22-33*) and therapeutic tissue ablation (*34*).

The present invention relates to the use of phenanthro[1,10,9,8-opqra]perylene-7,14-dione (Fig. 1A) derivatives as necrosis-avid contrast agents. Typical examples of such derivatives are the natural photosensitizing and photosensory pigments like hypericin (Fig. 1B),

20   pseudohypericin (Fig. 1C), stentorin (Fig. 1D), the fringelites (Fig. 1E), the gymnochromes (Gymnochrome B (Fig. 1F), Gymnochrome D (Fig. 1G), Isogymnochrome D) and blepharismin (P.S. Song, 1995, *J Photoscience* 2, 21-35).

SUMMARY OF THE INVENTION

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The present invention is based on the unexpected finding that the use of a radiolabeled phenanthro[1,10,9,8-opqra]perylene-7,14-dione   derivative,   more   particularly   mono-[^{123}]iodohypericin allowed the *in situ* visualistion of infarcted, ischemic or necrotic tissue. Therefore, the main object of the present invention relates to the use of phenanthro[1,10,9,8-

30   opqra]perylene-7,14-dione derivatives as necrosis or infarct specific agents.

DETAILED DESCRIPTION OF THE INVENTION

List of Figures

35   **Figure 1:** Structure of phenanthro[1,10,9,8-opqra]perylene-7,14-dione and of different natural occuring pigments derived thereof: A: phenanthro[1,10,9,8-opqra]perylene-7,14-

dione, B: hypericin, C: pseudohypericin, D: stentorin, E: the fringelite D, F: Gymnochrome B, G: Gymnochrome D.

Figure 2: Chemical structure of hypericin (R = H) and mono-[^{123}I]iodohypericin (MIH) (R = ^{123}I).

5   **Figure 3:** Ex vivo images of rat liver obtained 30 min p.i. of MIH.

A. intact liver: (1) autoradiography of liver slice; (2) TTC staining of adjacent tissue block; (3) H&E staining of the same liver slice.

B. necrotic liver: (1) autoradiography of liver slice; (2) TTC staining of adjacent tissue block; (3) H&E staining of the same liver slice

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Figure 4: Ex vivo images of rat liver obtained 24 h p.i. of MIH

A. intact liver: (1) autoradiography of liver slice; (2) TTC staining of adjacent tissue block; (3) H&E staining of the same liver slice.

B. necrotic liver: (1) autoradiography of liver slice; (2) TTC staining of adjacent tissue block ;

15   (3) H&E staining of the same liver slice.

Figure 5: SPECT images (coronal section) obtained at 24 h (left) and 48h (right) post injection of MIH in a rabbit with myocardial infarction. The radioactivity can be persistently found in the heart region and to a lesser extent in the thyroid and gut.

Figure 6: Ex vivo images of rabbit heart. After TTC staining of a 5-mm thick slice (A), a 50-
20   μm frozen slice (B) and corresponding autoradiographic image (C) were obtained and matched when overlapped (D).

Description

The present invention is based on the surprising finding that hypericin, has a preferential
25   distribution and retention in infarcted, ischemic or necrotic tissue following the adminstration thereof to a human or animal subject. Hypericin is a naturally occuring photosensitizer, which belongs to the group of pigments derived from the fundamental chromophore phenanthro[1,10,9,8-opqra]perylene-7,14-dione. Next to synthetic derivatives of hypericin, this group further comprises other naturally occuring pigments such as stentorin, the
30   fringelites, the gymnochromes, and blepharismin. Therefore, a first object of the present invention is the use of compounds derived from the phenanthro[1,10,9,8-opqra]perylene-7,14-dione as for the manufacture of an imaging agent for obtaining an image of ischemic, infarcted or necrotic tissue. In a preferred embodiment said compounds have photosensitizing properties. In a more preferred embodiment said compounds are selected
35   out of the group of the naturally occurring pigments consisting of hypericin, pseudohypericin,

stentorin, the fringelites, the gymnochromes, and blepharismin and derivatives thereof. In a most preferred embodiment the invention relates to the use of hypericin or a derivative thereof as necrosis or infarct specific agent.

It is an aspect of the invention to use the said phenanthro[1,10,9,8-opqra]perylene-7,14-
5   dione derived compounds as necrosis-avid contrast agents as imaging agents in nuclear medicine imaging. In a more preferred embodiment, a radiolabeled derivative of hypericin is used. For imaging purposes any of the well-known medical radionuclides can be used as radiolabel. Suitable radionuclides include Tc-99m, I-123, I-125, I-111, In-113m, G-67, or other suitable gamma-emitters. Preferably radio-labeled hypericin is used, more preferably
10   hypericin is labeled on carbon 2 atom, in ortho-position of the phenolic group with the most acidic characteristics.

The person skilled in the art is aware that imaging techniques other than nuclear imaging are used for the purpose of visualizing ischemic or infarcted tissue, such as X-ray and magnetic resonance. The use of a contrast agent in an X-ray procedure requires that the agent is
15   labeled with a radiopaque material. Suitable radiopaque materials are well known and include iodine compounds, barium compounds, gallium compounds, thallium compounds, and the like. Specific examples of radiopaque materials include barioum diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric
20   acid, iosulamide meglumine, iosumetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone and thallous chloride. Labels that can be detected or that enhance the effects of magnetic resonance imaging include gadolinium, copper, iron and chromium. It is preferred that these metal atoms be prepared in the form of a conventional organometallic
25   chelates, which are then bound to the contrast agent.

In a second object, the present invention provides a method for the imaging of ischemic or infarcted tissue. Said method comprises the parenteral administration of an effective amount of an appropriately labeled contrast agent of the present invention to an animal or human subject. The dosage may vary depending upon the imaging technique used, the area and tissue to be imagined, the age and condition of the subject and other factors, which a skilled
30   practitioner would consider. After injection, sufficient time is allowed for the said imaging agent to accumulate at the site of the diseased tissue. In a preferred embodiment the method of the present invention, radiolabeled hypericin is injected parenterally, preferably intravenously, into a subject. After allowing sufficient time the subject is scanned with a
35   gamma camera.

Illustrative embodiment

Materials en Methods

Synthesis and preparation of mono-[^{123}I]iodohypericin

5   MIH was synthesized in no-carrier added form using a standard electrophilic radioiodination method in the presence of peracetic acid and was purified with high pressure liquid chromatography (HPLC) as described previously (37). After purification the MIH solution was evaporated to dryness at 60 °C under a flow of nitrogen and the tracer agent was redissolved in water/polyethylene glycol (PEG) 400 (8:2, V/V) immediately before injection.

10   *Animal models of Necrosis*

All animal experiments were conducted with the approval of the institutional ethical committee.

1. **Reperfused hepatic infarction**: six adult Wistar rats weighing 400-450 g were anesthetized with intraperitoneal injection of pentobarbital (Nembutal®, Sanofi Santé

15   Animale, Brussels, Belgium) at a dose of 40 mg/kg. Under laparotomy, reperfused hepatic infarction was induced by temporarily clamping the hilum of the right liver lobes for 3 hours. After reperfusion by declamping hepatic inflow, the abdominal cavity was closed with 2-layer sutures and the rats were allowed to recover for 8h after the surgery (*19,37*).

20   2. **Occlusive myocardial infarction**: A New-Zealand adult rabbit weighing about 5 kg was sedated with intramuscular injection of a mixed solution of Ketalar (ketamine hydrochloride, Pfizer, Brussels, Belgium) at 15 mg/kg and Rompun (xylazine hydrochloride, Bayer, Leverkusen, Germany) at 2.5 mg/kg. The animal was then anaesthetized with an intravenous bolus injection of Nembutal at 15 mg/kg followed

25   by IV infusion of Nembutal at 0.1 mg/kg/min through an ear vein. Under a cold-light laryngoscope with No. 1 pediatric blade (Riester®, Jungingen, Germany), the animal was intubated with a 3.5-mm endotracheal tube, which was then connected to an artificial ventilator (Mark 7 respirator, Bird Corporation, Palm Springs, CA). An open chest surgery of left posterolateral thoracotomy was performed. The pericardium was

30   opened to expose the left circumflex coronary artery (LCX). A loose snare loop of a 3-0 silk suture was applied around the artery and tightened with ligature. The chest was closed after evacuation of the pneumothorax.

Tracer administration

Five hours after reperfusion of the hepatic infarction, rats were intravenously injected with 3.7 MBq of MIH through a tail vein.

Two hours after reperfusion of the myocardial infarction, the rabbit was intravenously
5   injected with 130 MBq MIH via an ear vein.

SPECT imaging:

The rabbit imaging measurements were performed using a two-detector gamma camera (E-cam, Siemens Medical Systems, Erlangen, Germany). The rabbit was secured to the head-holder of the patient bed with the organ of interest in the center of the field of view. SPECT
10   projections were collected using the following acquisition parameters: 120 projections over 360° per detector head (step & shoot mode), 25 s per projection, a matrix size of 256x256, a pixel size of 2.4 mm, and a non-circular orbit. The images were reconstructed with maximum-likelihood expectation-maximization, applying 47 iterations.

In vivo SPECT was conducted under the same anesthetic regimes as that for surgery of the
15   rabbit. The rabbit was imaged at 24 and 48 hours after injection of MIH.

Animal Sacrifice, Tissue Sampling and TTC staining

Thirty minutes (n=3) or 48 hours (n=3) post-injection, the rats were sacrificed with an intravenous overdose of Nembutal. The rats were dissected and the organs were weighed and counted in a gamma counter (Wallac Wizard, Turku, Finland). Normal (dark red) and
20   necrotic (pale) parts of the liver were prelevated for either tissue counting or autoradiography and tissue staining For the latter two techniques, tissues were quickly frozen in isopentane at -50°C.

For the rabbit experiment, the heart was cut perpendicularly to the long axis into 5-mm thick slices starting from the apex. The slices were immersed in a buffered triphenyltetrazolium
25   chloride (TTC) solution for staining during 10 min at 37°C. From one slice, samples of normal and infarcted myocardium (guided by TTC staining) were weighed and counted in a gamma counter. Another slice was quickly frozen in isopentane at -50°C.

The liver tissue chunk remaining after obtaining frozen sections for autoradiography, was immersed in a TTC solution for staining during 10 min at 37°C. The tissue chunk was then

placed on a transparant foil with the sectioned surface facing down and scanned with an optical scanner (Hewlett Packard ScanJet 5400C). After this staining, normal liver and myocardium tissues appeared brick red and the necrotic areas were unstained (23,25,28-33).

5   *Ex vivo autoradiography and H&E staining*

The frozen liver and heart tissues were cut at -20°C into 20-50 μm serial sections and exposed overnight to a high performance storage phosphor screen (Super resolution screen, Canberra-Packard, Ontario, Canada). The screen was read using a Phosphor Imager scanner (Cyclone™, Canberra-Packard). The images were analyzed using Optiquant™

10   software (Canberra-Packard). Afterwards, these same sections were stained with haematoxylin-eosin (H&E) using the conventional procedure. These H&E stained sections were digitally scanned and the obtained images were corrected for brightness and contrast.

Tissue Radioactivity Counting

Guided by TTC staining, the liver lobes of the rat and heart sections of the rabbit were

15   sampled for normal and necrotic parts, weighed separately and counted for radioactivity using a 3-in. NaI(Tl) scintillation detector mounted in a sample changer (Wallac Wizard, Turku, Finland). Corrections were made for background radiation and physical decay during counting. The activity in the normal and infarcted parts of the liver and heart was then expressed as counts per gram tissue.

20   **Results**

Liver lobe infarction in rats

Tissue radioactivity counting. At 30 minutes p.i. the ^{123}I concentration is high in the blood, the spleen, the liver and the lungs. The concentration in the intact liver lobe is 2 times higher then in the necrotic liver lobe. Due to the clearance to the bile, the concentration of ^{123}I at 24

25   h p.i. increases in the small and large intestine and decreases in the intact liver whereas it remains almost unchanged for the necrotic region. The thyroïd concentration is quite high indicating deiodination. (Table 1)

Ex vivo autoradiography and histochemical Staining. Figure 3 and 4 compare the outcomes of autoradiography, H&E and TTC staining at respectively 30 min and 24 h p.i. of MIH. The

30   slices of intact liver obtained at 30 min p.i. (Fig. 3) and 24 h p.i. (Fig. 4) both show a

homogeneous deep red TTC staining (A2) and purple H&E staining (A3) confirming the viability of the tissue. Pronounced radioactivity uptake with a dotted distribution at 30 min p.i. was observed on the autoradiography of the slice of the intact liver (Fig 3, A1). At 24 h p.i. activity from the intact liver has cleared (Fig 4, A1).

5   The slices obtained from the infarcted liver lobe at 30 min p.i. (Fig. 3) show a low uptake of MIH (B1) in regions which appear light red on TTC staining (B2) and pink after H&E staining indicating necrosis (B3). In some regions a high uptake of MIH with similar distribution as in the intact liver slice is observed. This region corresponds to regions stained deep red with TTC and purple with H&E, indicating that these regions are viable. It should be noted that
10   the low contrast between viable and necrotic tissue after TTC staining is due to the early timepoint after induction of infarction and that the shape of the TTC stained specimens deviates from the autoradiograms and H&E slices as the former is obtained after thawing the tissue from which the slices of the latter are obtained in frozen conditions.

The slices obtained from the infarcted liver tissue at 24 h p.i. (Fig. 4) show a relatively high
15   uptake of MIH (B1) in regions which are stained pink by TTC (B2) and pink-purple (B3) by H&E confirming necrosis. In regions which are stained deep red (viable tissue) or pale (no reperfusion) by TTC, corresponding to respectively pink or purple regions after H&E staining, absence of radioactivity is observed on the corresponding autoradiogram

Myocardial infarction in the rabbit

20   *In vivo SPECT imaging:* Twenty four and 48 hours post injection of MIH in the rabbit with occlusive myocardial infarction, the coronal section view after iterative reconstruction of whole body SPECT persistently displayed only one hot spot in the anatomical area of the heart. To a lesser extent, uptake of activity could also be seen in the regions of thyroid and intestines (Fig. 5).

25   *Tissue radioactivity counting:* TTC staining enabled selective sampling of reddish normal myocardium and whitish infarcted myocardium for radioactivity quantification. The results showed a 7-fold higher radiotracer concentration in the infarcted tissue than in the healthy tissue.

Ex vivo histochemical staining and autoradiography: Figure 6 compares TTC staining of a 5
30   mm thick slice, a 50-µm frozen slice with the corresponding autoradiographic image and a superposition of the two images. On the TTC stained specimen with right ventricular wall attached (Fig. 6a), the transmural infarction involves the entire anterior and lateral wall of the

left ventricle including the posterior papillary muscle (negative staining with TTC). On the frozen slice with right ventricular wall removed (Fig. 6b), the normal myocardium at the posterior wall and interventricular septum are stained only superficially with TTC (by the nature of this macroscopic staining technique). On autoradiography (Fig. 6c,d), a "doughnut"
5   pattern of radioactive uptake appears only in the infarcted region. However, the highest activity (in red) is found mainly subendocardially and near the lateral border zone, a sign characteristic for occlusive myocardial infarct (39,40).

Discussion

As hypericin is a polyphenolic polycyclic quinone, it can be labelled efficiently and quite
10   simply with radioiodine by electrophilic substitution in ortho position of a phenol (37). Structural analysis of the radioiodinated derivative has shown that in this way one iodine-123 radionuclide is reproducibly introduced on carbon atom 2, in ortho position of the phenolic group with the most acidic characteristics (Fig. 2). The resulting mono-[^{123}I]iodohypericin (MIH) could efficiently be separated from the starting material hypericin by reversed phase
15   HPLC and was obtained with an over 99% purity in non-carrier added form.

HPLC purified MIH was injected in rats with partial reperfused liver infarction.

Thirty minutes after intravenous injection of MIH in rats, still a high concentration of ^{123}I is present in the blood and MIH shows a pronounced uptake in the liver, the spleen and the lungs (Table 1). At 30 min p.i. the concentration in intact liver is twice as high as the
20   concentration in the necrotic liver lobe. MIH is a lipophilic compound which is cleared from plasma by hepatobiliary excretion (37, 41) and this results in a high concentration in normal liver at 30 minutes and a secretion of the activity via the bile to the gastrointestinal tract. This results in a lower concentration in the blood, the lungs, the spleen and the intact liver and an increased concentration in the GI tract at 24 h post injection. The high concentration
25   in the thyroid indicates that in vivo deiodination of MIH occurs. In contrast to the intact liver, the concentration of ^{123}I in the necrotic liver remains almost constant between 30 minutes and 24 hours post injection and at the latter time point the concentration in the necrotic liver tissue is 3.2 times higher than in the intact liver tissue. Since it can be assumed that the hepatocyte transporter systems of the necrotic liver lobe will not be functional anymore, the
30   mechanism of retention of MIH in the necrotic liver lobe will be different from that in the intact liver tissue. At 24 hours p.i., the concentration ratios of necrotic liver vs. blood and necrotic liver vs. muscle are very high (respectively 17 and 57).

Autoradiography images of the control and the infarcted liver tissue obtained at 30 min p.i. shows a "dotted" distribution of MIH in accordance with the typical lobular arrangement of

the intact liver tissue. At 30 min p.i. the uptake in the necrotic liver tissue is clearly lower than in the intact liver tissue (Fig. 3) , both images have the same scale), but some parts of the infarcted lobe still show the same dotted appearance as the intact tissue. Close examination of the corresponding TTC staining of the infarcted tissue obtained at 30 min p.i.

5   shows that the zone with high uptake on autoradiography is dark red after TTC staining whereas zones with low activity on autoradiography are pink after TTC staining. This indicates that the infarcted lobe still contains viable tissue (confirmed by TTC staining) which still actively extracts MIH from blood besides infarcted tissue in which relatively little MIH is retained.

10   At 24 h post injection the distribution pattern on autoradiography is the inverse of the image obtained at 30 min. The necrotic tissue now shows a higher concentration of ^{123}I distributed in a patchy fashion, whereas the intact liver tissue from which the MIH has cleared to the bile is now devoid of radioactivity.

        Again in some animals the autoradiography showed a heterogenous activity distribution with
15   low concentration in zones which after TTC staining are either red (viable tissue) or white (tissue lacking reperfusion) whereas zones which are pink on TTC staining are characterized by a relative high concentration of radioactivity. Comparison of the autoradiography image of the necrotic tissue obtained at 24 h p.i. with the image obtained after haematoxylin-eosin staining of the same tissue slice, shows that areas with high concentration of radioactivity
20   are pink-purple coloured whereas areas with low radioactivity concentration either are pink (tissue lacking reperfusion) or purple (viable tissue coloured after H&E staining confirming that uptake of MIH is confined to reperfused areas which are necrotic.

        The rat model of liver necrosis is relatively easy to set up yet it is not ideal for the evaluation of MIH as this tracer is cleared from the plasma by hepatobiliary excretion which complicates
25   interpretation of the MIH liver images.

        Therefore we have done an additional experiment using a rabbit model of myocardial infarction which is instrumentally more challenging but provides cleaner images as MIH does not appear to accumulate in the normal myocardium in neither mice or rats.

        SPECT imaging after injection of MIH in the rabbit model shows a hot spot in the heart
30   region, both at 24 h and 48 h post injection (Fig. 5 and 6).

        The rabbit heart was excised and stained with TTC allowing to discernate the normal (deep red) from the infarcted tissue (pale pink).

        Quantification of the concentration of MIH obtained by tissue counting shows a 7 fold higher uptake in the infarcted region (0.36 % of ID/g) compared to the normal region (0.05% of
35   ID/g). The ratio is higher than the ratio observed for the rat liver model as in contrast to

intact liver, the intact myocardium does not accumulate MIH and because a later timepoint (48h vs 24 h) was used in the rabbit experiment. The myocardial distribution of MIH was visualized with autoradiography and, in accordance with the rat study, a nice correlation was found between the areas not stained by TTC and the areas with higher uptake of

5   radioactivity (Fig. 6). The autoradiography image shows that most of the MIH is found subendocardially suggesting that an occlusive myocardial infarct was induced in the rabbit so that delivery of MIH was restricted to diffusion from the ventricle attaining only the lateral border zone of the subendocardium. Quantification of the autoradiography image shows an uptake ratio of about 6 (comparable with the ratio observed after gamma counting) for the

10   overall infarcted myocardium vs. the intact myocardium. When the region of the infarcted myocardium is limited to the subendocardial area, an uptake ratio as high as 16 is found for the infarcted tissue vs. the control tissue.

Regarding the mechanisms behind the observed necrosis-avidity of MIH it may be hypothetised that the mechanism is similar to the retention of hypericine in tumoral tissue. It

15   is known that both hypericine and MIH bind extensively to HDL and LDL *in vivo* (37). In tumoral tissue it was found that the hypericine-lipoproteine complex extravasates in the tumor as a result of the higher permeability of the tumor vessels. Hypericine then leaves the lipoprotein and binds to the either collagen or cellular membranes.

Also in reperfused infarcted myocardium elevated permeability of the microvasculature is

20   observed (42), so that MIH may use the same retention mechanism as hypericine in tumoral tissue. In detail studies are however required to confirm this hypothesis.

The present preliminary study has shown that MIH has necrosis avid properties and merits further evaluation as a potential useful tracer agent for tissue viability assessment. It is clear, however, that more investigations are needed, especially with respect to the dynamics of

25   uptake, the earliest time-point when necrosis becomes visible and the mechanism of uptake in comparison with earlier reported necrosis avid tracer agents such as Tc-pyrophosphate and Tc-glucarate. In addition, further chemical optimization of this tracer appears to be indicated. The thyroid uptake observed after injection of MIH in both the rat and the rabbit suggests *in vivo* deiodination. For this reason and in view of the optimal characteristics of

30   technetium-99m, a labeling with ^{99m}Tc would be more appropriate and will be included in further studies where MIH will serve as a lead compound.

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Tables

Table 1. Biodistribution of MIH in rats expressed as % of injected dose per gram tissue (mean±SD, n=3)

5

	Time p.i.	
	30 min	24h
Blood	1.34±0.38	0.10±0.01
Stomach	0.03±0.00	0.37±0.13
Small intestine	0.09±0.03	0.27±0.02
Large intestine	0.03±0.01	1.47±0.13
Kidneys	0.35±0.05	0.18±0.02
Spleen	2.19±0.18	0.43±0.07
Normal liver	3.80±1.39	0.55±0.15
Necrotic liver	1.91±0.46	1.71±0.32
Heart	0.47±0.13	0.24±0.08
Lungs	1.60±0.58	1.06±0.30
Muscle	0.03±0.01	0.03±0.00
Thyroid	0.16±0.02	9.25±3.25
Necrotic liver/Intact liver	0.57±0.28	3.18±0.49
Necrotic liver/Blood	1.44±0.10	17.40±1.19
Necrotic liver/Muscle	75.80±16.34	57.09±4.24